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By David Blankenship

ORIGINAL

#16 Petition

PATENT

Attorney Docket No. 016994-003122

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of

N. DOROFF, ET AL.

Serial No.: 08/154,019

Filed: November 16, 1993

Examiner: J. Chambers

Art Unit: 1804

For: PRODUCTION OF RECOMBINANT POLYPEPTIDES BY BOVINE SPECIES AND TRANSGENIC METHODS

DECLARATION OF NEAL FIRST UNDER 37 CFR §132

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Neal L. First, state as follows:

1. My present position is Professor of Reproductive Biology and Animal Biotechnology at the University of Wisconsin. I am a consultant for several companies including Pharming B.V., the assignee of the above-captioned application (the '019 application). A copy of my curriculum vitae is attached. I have been asked by Pharming B.V. to give my opinion of the claims to methods of generating transgenic bovines in the '019 application in view of the comments in the office action mailed May 2, 1995.

2. In forming my opinion, I have reviewed the '019 application, the office action mailed May 2, 1995 and pertinent references cited therein. One of these references is First, US 5,231,979 (the '979 patent), of which I am the first-named inventor.

3. I note that the '019 application is directed (in part) to methods of producing transgenic bovines (hereafter the Pharming methods). The methods involve the following steps: harvesting immature oocytes from bovines, culturing the immature oocytes in vitro, fertilizing the oocytes in vitro to produce

zygotes, introducing a transgene into the zygotes, culturing the zygotes *in vitro* to form a embryo and transplanting the embryo into a female bovine. The application also exemplifies the successful use of the methods to produce a transgenic bovine.

4. I understand that the Examiner takes the position that the successful application of the Pharming methods to achieve a transgenic bovine would have been obvious from the '979 patent. I respectfully disagree with this position for the reasons stated below. To the contrary, I believe that the successful practice of the Pharming methods represents a substantial advance in the art whose attainment was not reasonably expected from, e.g. the '979 patent.

5. The Pharming methods represent a substantial advance in the art because quite surprisingly they made possible a greater range of genetic manipulations than that performed in the '979 patent. The '979 patent discusses the development of an *in vitro* culturing method that allowed production of viable bovine blastoysts. The '979 patent of First et al. is directed to generic methods for the culture and co-culture of bovine embryos.

The DeBoer et al. '019 application is not directed to a method of culturing bovine embryos per se, but rather uses the method in development of a system for producing transgenic cattle. It is the system and its successful application that is novel and inventive for cattle in the DeBoer application

Our goal underlying the method in the '979 patent was the manipulation of blastocyst-stage embryos (as described in column 1, lines 55-60). For example, this procedure would expedite the generation of herds of genetically superior animals (particularly dairy cattle) by allowing cloning of blastocyst believed to have desirable naturally occurring characteristics. We did not perform any manipulations involving introduction of transgenes into embryonic cells. By contrast, the Pharming methods allow introduction of any transgene into a zygote leading to phenotypes

not found in nature, such as a cow producing milk containing a human protein.

At the time of filing the '979 patent we did know that transgenesis was possible in mice and sheep and we thought it might some day be possible in cattle. Thus, in the '979 patent, we noted that *in vitro* culture might be used in genetic engineering (column 1, line 62). However, we did not describe particular transgenesis procedures (e.g., embryonic stem cells, microinjection of zygotes or infection with retroviruses) or indicate how our *in vitro* culture method might be adapted to be exploited in any of these procedures.

6. In may opinion, the successful practice of the Pharming methods could not reasonably have been expected from the method described in the '979 patent. This opinion is based in part, on the following facts.

(a) The scientific literature as of December 1989 (the effective filing date of the above captioned application) indicated that attempts to produce transgenic bovines up to then had proved extremely difficult, lengthy and expensive. For example, one review article reports:

> Most scientists working on transgenic animals--be it for improving traits such as feed efficiency or for using them as factories for human pharmaceuticals--shy away from cattle. In the cow...you have a three-four year project. And it's a costly venture as well

Van Brunt, *Bio/Technology* 6, 1149-1154 (1988) at p 1152

As of December 1989, there were no confirmed reports in a peer-reviewed scientific journal of any viable transgenic bovine calf having been produced. The Biery, Lostkutoff and Bondioli references mentioned in the office action discuss only attempts which did not generate transgenic bovine calves. Although a very low frequency of expression was obtained in early fetuses. Today expression in embryos and early fetuses is known to often be from non chromosome integrated DNA (Krisher et al., *Animal Biotechnology* 6, 15-25 (1995); Bowen et al., *Biol. Reprod.* 50, 664-668 (1994)). In light of this general background of failure

and frustration, most practitioners (including me) would have approached alternate methods of generating transgenic cattle with a measure of skepticism and an expectation that considerable empirical experimentation lay ahead.

(b) There would have been a number of problems and uncertainties in trying to combine the *in vitro* procedure with the poorly successful bovine methods of Biery, Loetkuto[illegible] and Bondioli, in which oocyte maturation and fertilization are performed *in vivo*, but transgenic offspring did not result. For example, it was unpredictable whether traditional microinjection procedures for *in-vivo* eggs could have been successfully applied without modification to *in-vitro* matured oocytes. The *in-vitro* matured oocytes might have different physiological properties (e.g., structural differences in the zona pellucida (the protein layer surrounding the oocyte, its hardening, etc.) due to the different environment in which maturation occurred; to my knowledge, this had never been thoroughly investigated in any species. It was also unpredictable whether the phasing of the cell cycle of *in-vitro* oocytes would have been different or impact on the visibility of the pronucleus and therefore on the injection protocol. Difficulty may also have related to the relative timing of microinjection and fertilization which might not be the same for *in-vivo* and *in-vitro* matured oocytes. The relative timing would have been expected to be important in development of a successful protocol, because if the DNA was injected before S phase, it might be degraded before integration and after S phase, it could not be integrated until the two cell stage.

(c) A further source of unpredictability was whether the block on bovine embryo development *in vitro* occurring at about the 8-cell stage could be overcome, and if so, with what efficiency, in the context of a transgenesis protocol. The 1990 patent describes how the block on bovine embryo development could be overcome at an efficiency of about 20% by supplementing culture media with epithelial cells in the context of the cloning protocol used. This was an empirical observation; at that time, we did not understand the mechanism by which the block occurred

or by which it was relieved. The [illegible] a manipulation (i.e., microinjection) that [illegible] impact on cell physiology (e.g. phasing of the cell cycle), it was unclear whether and to what extent epithelial cell supplementation of culture medium would be effective in [illegible] the cell blockage at the eight-cell stage. Today there are totally defined media culture systems effective in culturing bovine embryos to blastocyst (i.e. Rosenkrans & First, J. Animal Sci. 72: 434-437 (1994)). This was not considered possible when the '979 patent studies were done.

[illegible] uncertainties discussed in the previous paragraph might have required considerable experimentation to perfect or overcome. Yet the ultimate end point for the efficacy of such experimentation (i.e., the production of a viable transgenic bovine) would not have been apparent until several years later, so that practically it was not possible to vary systematically most of the parameters. These factors explain my view that the provision and demonstrated efficacy of Pharming's transgenic methods were not a routine development.

8. That others hold similar views regarding the substantial and dramatic advance of the Pharming's method in a difficult and unfruitful field is illustrated by the following comments:

> The commercial development of transgenic bovine technologies, however, has been frustrated because the protocols used successfully with smaller animals--which require large numbers of embryos and several surgical procedures-are prohibitively expensive when applied to cattle. The establishment of an *in vitro* embryo production system, as described by Herman de Boer and his coworkers at Gene Pharming Europe...is therefore a dramatic breakthrough in enlarging the transgenic pharm-yard.

[illegible], 5 (1994):

> A group in the Netherlands reports in a paper in the September issue of *Bio/Technology* successful generation of the first transgenic dairy cattle.

which carries a gene for production in cow milk of human lactoferrin (hLF). Confirmation that the gene integrated in vitro [illegible] for generation of transgenic cattle.

Selzer, *Chemical & Engineering News* 6?, ? (1993)

But historically, efforts to produce transgenic dairy cows have been thwarted because of cumbersome and costly surgical procedures. Now however, researchers from Gene Pharming Europe have circumvented the need for surgical removal and transfer of embryos by combining gene transfer with an *in vitro* embryo production system.

10. Finally, I wish to state for the record that the purpose of this declaration is to assist in assessment of the patentability of the Pharming claims and the validity and [illegible] of the claims in the 1979 patent involve different issues.

I have been duly warned that willful false statements and the like are punishable by fine and imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified patent application or any patent issuing thereon.

Neal I. First

Nov. 8 1995

Dated